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COMPARISON OF REFRACTIVE INDEX ESTIMATED FROM SINGLE-CELL AND BULK OPTICAL PROPERTIES

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ABSTRACT

Particle size and refractive index distributions are important determinants of bulk optical properties in the ocean. We have compared refractive indices of phytoplankton cultures estimated from individual particle and bulk optical measurements. For individual particles, size and refractive index were estimated from flow cytometric forward and side scattering measurements combined with Mie theory. Using an inverse method, bulk refractive index was estimated from cell concentration, measured size distributions, absorption, and attenuation. For several cultures, phytoplankton refractive index estimated from the inverse method was lower than that estimated from flow cytometric scattering measurements. These differences appear to be caused by higher side scattering than predicted by Mie theory, most likely due to deviation from the assumption of homogenous spheres. Using a diameter correction factor determined for ten species of phytoplankton, we have developed a methodology that works well for determining size and refractive index of cells in the laboratory and that can be applied to natural particles. Examples of flow cytometric results for particles from New England continental shelf waters are presented.

INTRODUCTION

Changes in the properties of particle pools are an important source of optical variability in the oceans. Temporally and spatially, there can be variability in the relative concentrations of different particle types and in individual particle properties, such as diameter and refractive index (real and imaginary parts, n and n′, respectively). Flow cytometry is one of the few tools available for analyzing the optical properties of large numbers of individual particles. Using flow cytometry, it is possible to distinguish among specific groups of particles in the ocean, including *Prochlorococcus*, *Synechococcus*, eukaryotic pico- and nanophytoplankton, coccolithophorids, pennate diatoms, cryptophytes, and heterotrophic bacteria (Olson et al. 1989, 1993; Marie et al. 1997). In previous work, particle size has been estimated based on empirical relationships between flow cytometric forward scattering and cell volume (Olson et al., 1989). Progress has also been made using a combination of Mie theory and flow cytometric forward and side scattering measurements to estimate both diameter and refractive index for individual particles (Ackleson and Spinrad 1988). We have found that the successful application of Mie theory to natural particles is dependent on a better understanding of how these particles deviate from the assumptions of sphericity and homogeneity inherent in the theory.

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APPROACH

We measured the abundance and optical properties of individual particles during the Coastal Mixing and Optics (CMO) experiment. The CMO experiment was focused on sampling of optical and physical properties at a single location (40°30' N, 70°30' W) off the U.S. northeastern coast. We participated in sampling during two three-week periods in late summer, 1996 and spring, 1997. Particles in 5-ml samples were analyzed with a modified Coulter EPICS flow cytometer in which each optical signal was split and measured by two photomultipliers at different gain settings, which increased the dynamic range of the measurements (allowing cells ranging in size from approximately 1 to 40 microns to be analyzed simultaneously).

In order to use Mie theory to estimate particle size and refractive index from our flow cytometric (FCM) data, we first developed an optimization routine to minimize the difference between side and forward scattering measured by FCM and estimated using Mie theory. Such an optimization is necessary because the optical geometry of the flow cytometer is not precisely known. We performed an unconstrained, nonlinear minimization for calibration particles using optimization routines provided with the MATLAB software package (Mathworks, Inc.).

There were four variables in the optimization, two of which were used to weight forward scattering values (fls1 and f1) and two of which were used to weight the side scattering values (ssc1 and f2). For our FCM setup, narrow beam obscuration bars run across the collection lens for both the forward and side scattering detectors and, additionally, the entire lower half of the lens for the forward detector is obscured. This geometry leads to a weighting function for the forward detector of:

$$w1(\theta) = 1\text{-}fls1*2/(\pi*d_l*\tan(\theta*2*\pi/360))$$

where $3° <= \theta <= 19°$, d_l = distance from particle to lens (= 24.9 mm), and fls1 = 1/2 width of obscuration bar. A sine function was used to represent the weighting for side angle scattering, using the following equation:

$$w2(\theta) = \sin(\pi*(wa*\ \theta+wb)/180)$$

where $ssc1 <= \theta <= (180\text{-}ssc1)$, $wa = 180/((180\text{-}ssc1)\text{-}ssc1)$, and $wb = -wa*ssc1$.

As in Ackleson and Spinrad (1988), differential scattering cross sections in the forward and side directions were calculated by multiplying the forward and side weightings, w1 and w2, by the first two elements of the scattering matrix [S11 and S12 in the notation of Bohren and Huffman (1983)], and summing over all angles for each detector. In our approach the additional factors, f1 and f2 (which were also determined in the optimization) are scaling factors necessary to determine the final differential scattering cross sections for forward and side angles, respectively.

In order to perform the optimization, we used FCM measurements of calibration particles of known refractive index and/or size. These particles included polydisperse oil suspensions of heptane (n=1.0325, relative to water), nonane (n=1.0467), and dodecane (n=1.0590), five sizes of polystyrene beads (0.66, 2.9, 3.79, 5.2, and 6.2 micron; n=1.19;

Polysciences, Inc.) and silica beads (1.58 micron; n=1.09; Duke Scientific, Inc.). Oil suspensions of heptane, nonane, and dodecane were made by adding a small amount of each oil to a syringe of filtered seawater and shaking vigorously to form a polydispersion of droplet sizes (Ackleson and Spinrad 1988). The addition of the silica bead to the optimization was particularly important, because it is within the refractive index range of phytoplankton cells. With our optimization procedure, the theory adequately represents the measured relationship between forward and side angle scattering for beads and oils (Figure 1).

Using this optimization, a 3-dimensional "lookup" table of theory-based solutions for particle forward scattering, side scattering, and absorption cross section was created over plausible ranges of particle diameter (0-15 microns), n (1 to 1.15), and n′ (0 to 0.02) for natural marine particles. To evaluate the applicability of these solutions, we compared them to results from analysis of numerous phytoplankton cultures. The cultures used ranged in size from 1 to 8 microns, and for each a suite of measurements were made, including cell concentration, forward and side light scattering, and chlorophyll fluorescence (flow cytometer, at 488 nm), cell size (Coulter Multisizer), absorption (spectrophotometer and ac-9), and attenuation (ac-9). Experimental details are described in DuRand et al. (2000). For comparison to theory-based estimates, independent determinations of refractive index at 488 nm were calculated from our measurements. The anomalous diffraction approximation (ADA; Van de Hulst 1957) was used to calculate efficiency factors for absorption and attenuation, and then n' and n were calculated through iteration (following equations in Morel and Bricaud 1986; Bricaud and Morel 1986). Assumptions of the theory include that the particles are homogenous and spherical, with a refractive index close to that of the surrounding medium, which is reasonable for these phytoplankton cells. For each particle, theory-based estimates of diameter, n′, and n were chosen by minimizing the difference between FCM measurements and values in the Mie-based Lookup Table (MBLT) of forward scattering, side scattering, and absorption cross section.

This MBLT method was also applied to selected Coastal Mixing and Optics field samples. In this case, for phytoplankton, absorption cross sections were estimated using an empirical relationship between FCM chlorophyll fluorescence and absorption based on more than fifteen species measured in the laboratory. Using the MBLT method, diameter and refractive index were estimated for each particle analyzed. Size and refractive index distributions were created by considering all particles in a sample.

RESULTS

Laboratory Comparisons

For phytoplankton cells measured in the laboratory, values of diameter and refractive index estimated from the MBLT were compared to the independent determinations of these values. We found that for the ten species of phytoplankton studied, cell diameters were underestimated and refractive indices were overestimated (Figure 3). One explanation for the cause of the underestimate in diameter is that phytoplankton cells deviate from sphericity and homogeneity. In an attempt to characterize the average effects of these deviations, we determined a second-order

polynomial regression between estimated and measured diameters (Figure 2). This fit shows a good correlation (r^2=0.97) given that ten species of phytoplankton were included, all of which have unique shapes and heterogeneities. The regression was then used to correct MBLT diameter, and the imaginary refractive index was recalculated. Each particle was re-run through the MBLT routine with diameter and absorption fixed at the corrected values and with minimization only for forward scattering and side scattering values. The results show better agreement not only for diameter but, as expected, for refractive index as well (Figure 3). Errors in n′ are well correlated with deviations in diameter, suggesting that diameter needs to be determined with a high degree of accuracy in order to estimate n′.

We tested our diameter correction procedure by applying it to independent data from a diel study of *Micromonas pusilla*. In this experiment, *Micromonas* refractive index did not change significantly over the diel cycle (DuRand et al. 2000). However, diameter did show significant changes, which were well resolved by our MBLT method with diameter correction (Figure 4; linear fit with r^2=0.89). While this modified MBLT method worked well for a phytoplankton species not used to determine the correction, we cannot yet be sure that it is applicable to all phytoplankton species and physiological conditions.

Field Example

We applied the modified MBLT method to natural samples from the Coastal Mixing and Optics Spring 1997 cruise. Size and refractive index distributions for eukaryotic pico/nanophytoplankton and for *Synechococcus* were compared in 10 m samples from three different days (Figure 5). For both eukaryotes and *Synechococcus*, we consistently observed n values in the range 1.02-1.08, as expected for phytoplankton (Morel and Bricaud 1986). These distributions contrast with others derived for natural phytoplankton samples using individual particle analysis, which typically exhibit higher values (Ackleson et al. 1988). This improvement for our results is most likely due to the diameter correction which we have applied based on our study of phytoplankton cultures.

The three days we examined represent distinct ecological periods during the onset of spring stratification. April 30 corresponds to pre-bloom, well-mixed conditions, May 6 to onset of stratification and chlorophyll at peak concentrations, and May 10 to a period later in the bloom with lower chlorophyll concentration (Sosik et al. 2000). Cell concentrations for both eukaryotes and *Synechococcus* were lowest before the bloom, and highest on May 10 after chlorophyll concentrations had begun to decrease. We found that mean n and n′ for *Synechococcus* were always lower and more constant than for eukaryotes. Interestingly, n for eukaryotes was highest on April 30, while n′ was highest on May 10, indicative of changes in either species composition or intracellular properties. In future work, we will examine this variability in more detail.

FUTURE DIRECTIONS

Our comparison of Mie theory-based estimated size and refractive index for numerous phytoplankton cultures examined in the laboratory has allowed us to determine a methodology applicable to studying cells in natural samples. The examples we show

here indicate that it is possible to estimate distributions of size and refractive index from individual particle optical measurements that are within the expected range for phytoplankton cells. We will apply our methodology to determining spatial and temporal changes in particle diameter, n, and n′ during both the summer, 1996 and spring, 1997 CMO cruises. Estimates of individual particle properties will be used to interpret variability in bulk inherent and apparent optical properties, which were measured concurrently with single particle properties.

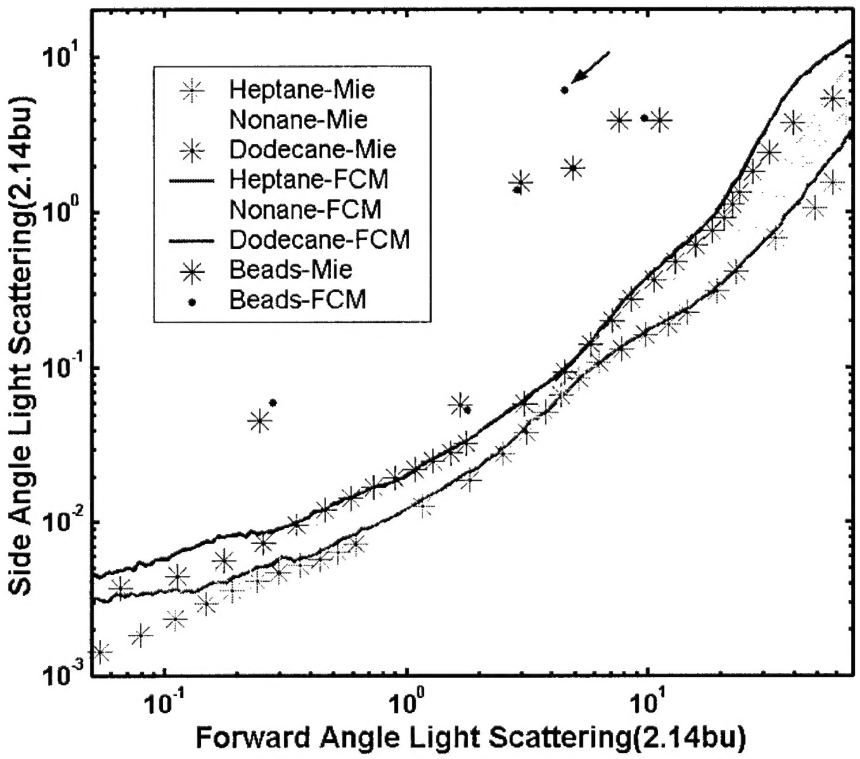

Figure 1. A comparison of theory-based estimates (stars) and flow cytometric measurements (lines and dots) of forward and side scattering for beads and oil dispersions. Several bead types were used in this comparison, including 0.66, 2.9, 3.79, 5.2, and 6.2 micron polystyrene beads and 1.58 micron silica beads. An arrow has been placed next to the 5.2 micron polystyrene bead which is the most poorly fit by this optimization. Oil dispersions of heptane, nonane, and dodecane are shown.

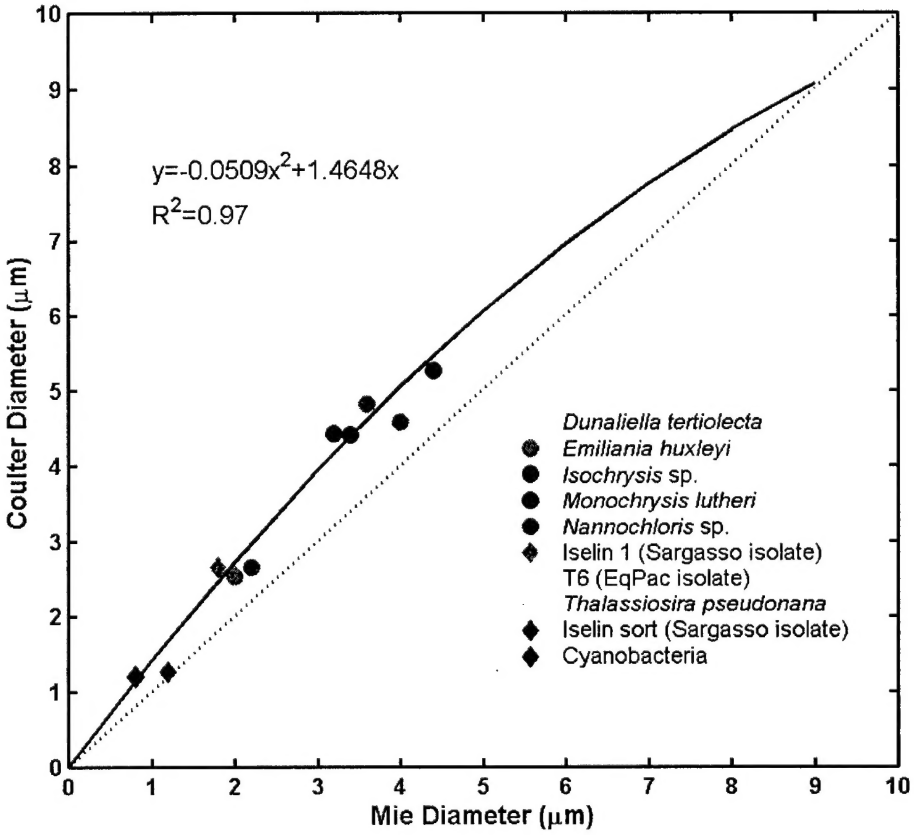

Figure 2. Polynomial relationship between theory-based estimates and Coulter measured diameters for a variety of phytoplankton cultures. The Coulter diameter plotted here is the diameter of the mean cell ($\overline{D}$) including effects of polydispersion.

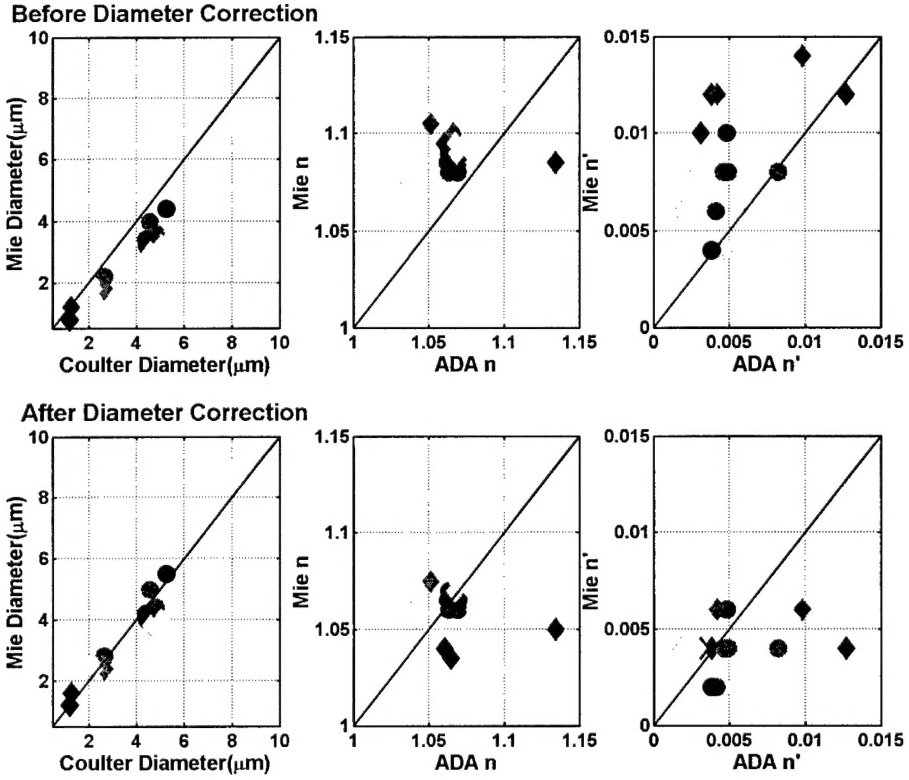

Figure 3. Comparison of cell properties estimated using the MBLT approach with independent estimates based on direct measurements of a variety of phytoplankton cultures. Uncorrected MBLT results are shown in the upper panels and the modified MBLT (including diameter correction) results are in the lower panels. Legend is the same as for Figure 2. Coulter diameters are the diameter of the mean cell ($\overline{D}$), and the ADA n and n′ are determined based on our measurements and the anomalous diffraction approximation. The value of ADA n > 1.10 for the cells from the Iselin sort is not considered to be plausible, as it is outside of the accepted range for phytoplankton cells.

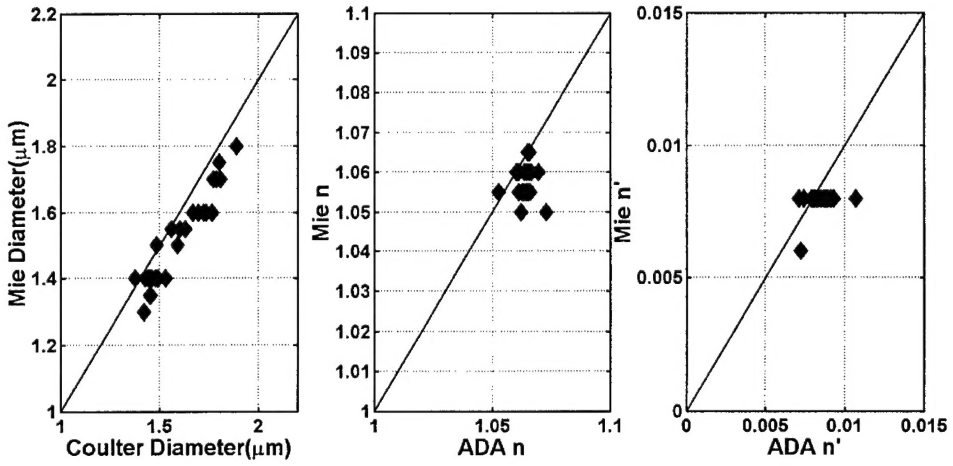

Figure 4. Comparison of cell properties estimated using the modified MBLT approach with independent estimates based on direct measurements of Micromonas pusilla sampled over a diel cycle.

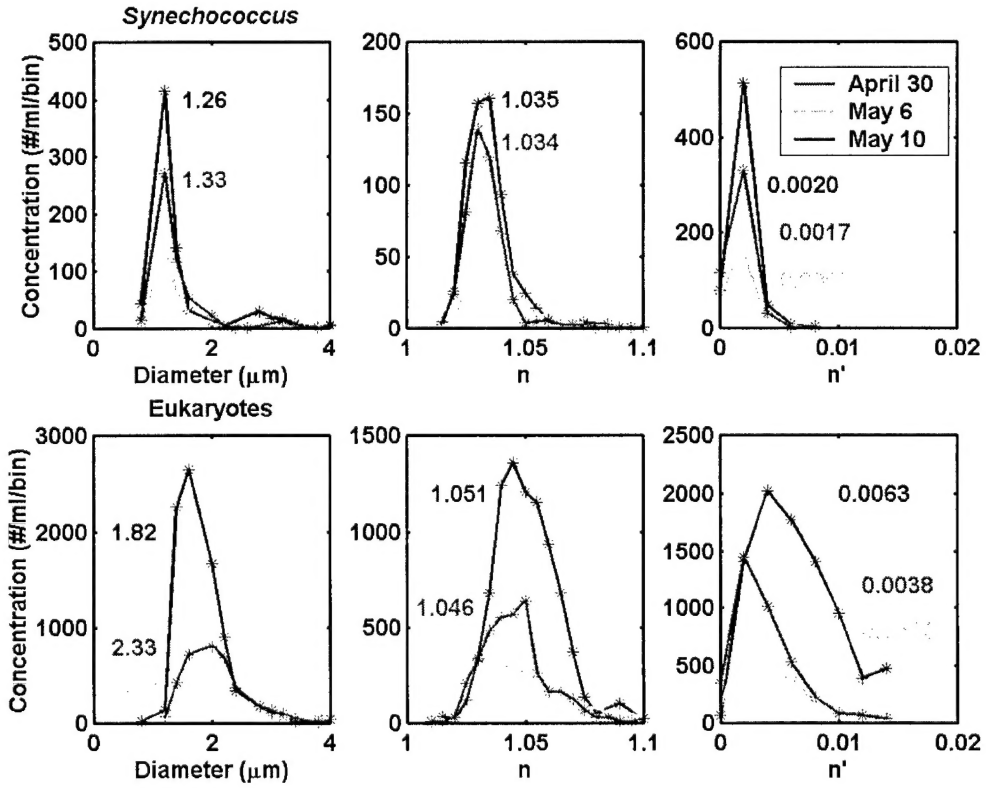

Figure 5. Size and refractive index distributions estimated with the modified MBLT method for three samples from 10 meters depth collected on different days during spring 1997. Mean values are indicated next to each distribution.

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